Special Report of the Technical Advisory Committee on Harmful Algal Outbreaks in Maryland: Causes and Significance of Menhaden Lesions

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Executive Summary

Members of Maryland's independent technical advisory committee on harmful algal outbreaks met together with regional experts in fish pathology and ecology to assess existing information regarding the causes of lesions found on menhaden and other fish, thought to be related to toxic outbreaks of Pfiesteria piscicida. In 1997 and 1998, lesions were found on only a small fraction of the young-of-the-year menhaden, particularly in smaller tidal rivers and creeks along the Eastern Shore and the Rappahannock and Great Wicomico Rivers in the Chesapeake Bay. The larger and deeper lesions found are ulcers developed as a result of fungal and bacterial infections and the defensive responses of the fish's cells. Fungal infections were not found on the smallest lesions and few fish collected from kills in which Pfiesteria was implicated have been examined for fungal infections. Consequently, Pfiesteria toxins, which have been demonstrated to erode the skin (epidermis) of fish in laboratory experiments, cannot be ruled in or out as initiators of fresh lesions or deep ulcers. The development of lesions is not required for Pfiesteria toxins to kill fish, consequently the uncertainty surrounding the causes of lesions does not call into question the linkages among fish kills, human health risks and toxic Pfiesteria outbreaks. This uncertainty does, however, mean that the prevalence of fish lesions alone should not be considered a reliable indicator of toxic Pfiesteria outbreaks. Development of molecular methods offers the promise of a more timely and reliable detector of Pfiesteria and its toxins for protection of public health. In addition, experimental research on the modes of fungal infection and progression of ulcer formation would help resolve the existing uncertainties regarding their relationships to Pfiesteria and improve our limited understanding of the effects of these maladies on fish populations.

Introduction

This is a special report of the Technical Advisory Committee (TAC) established in July, 1997, by Natural Resources Secretary John R. Griffin to advise the state agencies on their efforts to monitor the occurrence and evaluate the causes of fish lesions and fish kills initially observed in the Pocomoke River and later in Kings Creek and the Chicamicomico River during the summer and fall of 1997. Three previous reports provided independent peer assessments of information collected by state agencies relative to the causes of these fish lesions and kills, including the role of the toxic dinoflagellate Pfiesteria piscicida and related organisms (hereinafter, simply referred to as Pfiesteria). They also offered advice concerning the State's ongoing monitoring and assessment activities. Each of these reports addressed a broad array of issues, including not only fish health but also detection of toxic dinoflagellates and water quality measurements. In addition to these full TAC assessments, two other special meetings were held under TAC auspices involving experts who addressed the relationship of nutrient inputs to toxic Pfiesteria outbreaks (October, 1997) and best methods to monitor non point source pollution from the land (May,

1998).
Since the third TAC report submitted on March 11, 1998, there has been more in-depth pathological research conducted on the lesions, particularly those observed in menhaden (although other fish species may also be afflicted) under conditions thought to be conducive to toxic outbreaks of Pfiesteria. In particular, researchers have reported that these lesions are consistently infected by invasive and pathogenic fungal parasites. These findings raised the possibility that Pfiesteria toxins may not cause or contribute to these lesions. Reports of these studies have been interpreted by some in the media and the general public to mean that the toxic Pfiesteria outbreaks did not occur and, therefore, that the rationale for pollution controls being put into place is without scientific basis. Even beyond this public policy issue, the causes of lesions are of more than academic interest. The incidence of lesions observed in field monitoring has been used as an indicator of a potential, ongoing toxic Pfiesteria outbreak, thus being considered as a factor in State decisions to close tidal rivers to fishing and boating.
On December 16, 1998, the TAC, in conjunction with the interagency Maryland Pfiesteria study team, convened a workshop in Laurel, Maryland, to foster the exchange of information and ideas among scientists working on fish pathology and Pfiesteria assessments. Dialogue was then facilitated among the TAC members and fish pathologists present in an attempt to develop consensus, define differences, and describe uncertainties concerning the present understanding of the causes of menhaden lesions and their potential relationship with Pfiesteria. This TAC report presents the results of these deliberations.

Observations from Previous TAC Reports

It should be pointed out that in each of its three previous reports, the TAC continuously pointed out the uncertainties regarding the causes of the lesions observed in the Eastern Shore rivers. In August 1997 the TAC reported that lesions could have multiple causes and that most lesions include infections by bacteria and fungi. In September 1997 the TAC reported that some lesions appear similar to those caused by Pfiesteria in laboratory studies, but that the form of the lesions are not uniquely diagnostic. Again, the Committee pointed out that bacteria and fungi may be causative agents or merely secondary invaders. In the March 1998 report it was concluded that although Pfiesteria was the probable cause of the fish kills, multiple factors could be involved in the onset and development of lesions. The TAC recommended that, as a priority, experimental research should be conducted to determine the degree to which lesions should be used as an indicator of toxic Pfiesteria outbreaks.

The Workshop

The workshop was well attended, with over 50 participants. Presentations were made by those responsible for the fish, water quality, and Pfiesteria monitoring, investigators of fish health from academic institutions and state and federal agencies in both Maryland and Virginia, and by scientists from North Carolina who have authored studies on the effects of Pfiesteria toxins on fish health. Virtually every key player was in attendance. After the presentations and responses to questions by each presenter, the members of TAC and those making presentations contributed to a facilitated dialogue organized around seven central questions, which had been distributed to the participants in advance. These questions were designed to draw out scientific conclusions

relevant to management, rather than address detailed scientific issues. Throughout these questions, "fish lesions" are intended to mean those lesions affecting menhaden and other co-occurring species in tidal rivers and creeks that could potentially indicate exposure to toxins from Pfiesteria and not other broadly distributed maladies, such as those on striped bass that are also subject of recent attention. The guiding questions are the following:

1. What does the scientific evidence tell us about the probable causes of initiation and progression of these fish lesions? What are the limitations and uncertainties of those conclusions?
2. Under what circumstances can toxins produced by Pfiesteria cause or initiate lesions? How rapidly do any such lesions grow and become invaded by pathogens?
3. To what degree are lesions responsible for or presage observed mortalities and errant behavior of fish, such as those observed in the three Maryland rivers during 1997? What separate causes might be responsible?
4. How reliable is the use of the incidence and gross pathology of fish lesions as indicators of recent or ongoing toxic outbreaks? What other evidence could be collected to make such diagnoses more reliable?
5. Under what environmental conditions was there an elevated prevalence of lesions? What causes might be inferred?
6. What is the evidence or inference that fish with extensive lesions may stimulate toxic outbreaks of Pfiesteria?
7. What research and monitoring could best resolve remaining uncertainties regarding the causes of fish lesions?

Consensus Interpretations and Uncertainties

Initiation and Progression of Lesions (Questions 1 and 2)

A small fraction of the population of young-of-the year menhaden in the Chesapeake Bay has been found with lesions. Older fish caught in the open Bay are seldom affected and a higher prevalence of fish with lesions were found in certain areas, including the tidal rivers of the Eastern Shore that experienced fish kills and tidal tributaries of the Rappahannock and Great Wicomico rivers in Virginia. In general, lower prevalence of lesions was observed in these areas in 1998 than in 1997.

The well developed lesions on menhaden collected from these areas in 1997 and 1998 and subjected to histopathological examination were ulcers (see glossary) often showing deeply invasive infections by aseptate fungi, similar to Apanomycetes invadans. Histologic examinations have revealed that granulomas (a cellular immunological response of the fish involving encapsulation of the invading pathogen by white blood cells) surround the penetrating fungal hyphae. This suggests that fungal infection and the associated cellular responses of the fish are at least major contributors to the gross erosion of tissue characterizing these ulcers. The earliest (smallest) lesions, which may be small petecchial hemorrhages, however, do not show this hyphal invasion or granulomas. This suggests either that the primary fungal infection is not clearly apparent through microscopy or that some other stress, pathogen, and/or abrasion initiates the lesions, which subsequently expand due to fungal infection. Bacteria such as Psuedomonas

flourescens and Aeromonas hydrophila were also isolated from menhaden lesions. Indeed, the bulk of scientific evidence available to date indicates that fungal and/or bacterial infections are usually secondary. There are, however, fungal agents considered to be primary pathogens, for example Aphanomyces astaci, which causes European crayfish plague.
Toxins released by Pfiesteria piscicida can cause edema and loss of the epidermis of menhaden and other fishes based on evidence from direct exposure in two different laboratories in North Carolina. In addition, less definitive observations of lesions have been made during large Pfiesteria-induced fish kills in North Carolina. Under experimental conditions, the loss of epidermis may be rapid and, at high toxin concentrations, may affect extensive portions of the body. Unfortunately, few acutely affected or erratically behaving specimens of menhaden from Chesapeake Bay environments have been histologically examined to determine whether their tissues or lesions harbor fungal hyphae. It is possible that lesions directly caused by Pfiesteria toxins could have occurred in those cases, but these would be expected to appear histologically different from the ulcers involving fungi.

Exposure to Pfiesteria toxins could also be involved in the initiation of ulcers by eroding the epidermis and by causing physiological stress (such as immunosuppression) that could facilitate invasion by fungal or bacterial pathogens. Other risk factors such as environmental contaminants, low pH, hypoxia, other microbial toxins, overcrowding, etc. could initiate a general stress response in these fish, indirectly contributing to lesion formation. At this point, Pfiesteria toxins or other stressors cannot be ruled out as initiators of menhaden ulcers that obviously developed as a result of the activities of invasive aseptate fungi or bacterial colonizers.
Consequently, the widely observed, deep ulcers (as distinguished from those suggested by North Carolina investigators to form rapidly on dead or clearly intoxicated fish) may be either completely unrelated to Pfiesteria toxins or may be the result of exposure to these toxins days or weeks before.

Relationship between Lesions and Mass Mortality and Errant Fish Behavior (Questions 3 and 6)
Although deep, penetrating ulcers are likely to result in low-level chronic fish mortality, lesions themselves are not believed to be the cause of simultaneous, acute mass mortality observed in a fish kill, nor are they believed to cause the errant behavior observed in menhaden. Fish kills related to Pfiesteria probably result from acute exposure to the toxins, which as mentioned above, can cause at least the loss of epidermis. Lesions on fish dying in an acute fish kill event may also be chronic, initiated by earlier sublethal exposure to Pfiesteria toxins, some other stressor or pathogenic invasion by fungi and bacteria.
An interesting hypothesis that has been offered is that the loss of blood or other tissues through chronic ulcers may trigger Pfiesteria to metamorphose into toxic phases and cause a fish kill. While it has been demonstrated that under laboratory conditions compounds released by fish without lesions can induce metamorphosis and toxin production, the hypothesis that ulcerated fish may make this more likely, although presently not supported with direct evidence, certainly merits testing.

Lesions as an Indicator of Toxic Pfiesteria Outbreaks (Question 4)
One cannot determine the cause of the lesions solely by their shape, location, or gross appearance.

Furthermore, as reasoned above, even after microscopical examination one cannot rule Pfiesteria in or out as a factor in initiating lesions. Moreover, deep ulcers could be the result of exposure to sublethal concentrations of Pfiesteria toxins days or weeks earlier, and even at another location. Consequently, the prevalence of lesions alone is not a reliable indicator of an ongoing toxic Pfiesteria outbreak.

It has been suggested that finding an increased percentage of fish with lesions in repeated sampling over a period of days could indicate a toxic Pfiesteria outbreak. However, there may be other reasons that could produce such a result, including greater susceptibility of fish with lesions to capture, their immobility relative to healthy fish, and other stressors.

In order to provide a more reliable diagnosis of ongoing toxic Pfiesteria outbreaks, prevalence of lesions should be accompanied by other evidence, such as significant fish kills unexplained by hypoxia or other extreme events, observations of errant fish behavior or narcosis, and the presence of Pfiesteria. However, at present Pfiesteria cannot be readily identified in the field or quick laboratory analysis, although light microscope examination of water samples can yield presumptive enumeration of dinoflagellates resembling Pfiesteria. This limitation underscores the need for a reliable chemical, molecular or immunological method to detect and quantify Pfiesteria toxin in the environment. Once developed, the determination of a toxic bloom should be possible in a relatively short time period (within a day or two). For human health reasons, however, decisions to close a river need to be made in a more timely fashion than current technology allows for a definitive confirmation of a toxic outbreak.

Conditions Favoring Elevated Prevalence of Lesions (Question 5)

Lesions are generally only found in young-of-the-year menhaden from relatively low salinity waters (3 to 15 psu) in smaller tributaries or tidal creeks. While these are environments known to be preferred by Pfiesteria (based on limited sampling to date in the Chesapeake Bay and more extensive sampling of similar environments in North Carolina), they typically harbor other stresses, such as the episodic low dissolved oxygen and runoff of land-based contaminants, and pathogens which may cause lesions.

Research and Monitoring Needs (Question 7)

Additional research and monitoring is needed to resolve the cause and progression of lesions, understand the effects of these maladies on fish populations, and determine the relationship of Pfiesteria toxins to lesions and fish health. Some priorities include the following:

1. Histopathologic diagnosis is needed of fish collected at the time of apparent acute Pfiesteria toxin exposure, as indicated by mass mortality or errant behavior.
2. Experimental research on the modes of fungal infection is required to resolve whether other stressors or incipient lesions are precursors of infection. These could involve direct challenges by exposure to fungal zoospores. Additional research is also required on the progression of fungal ulcers that couples controlled experiments and field observations. This would help determine the environmental conditions most conducive to Aphanomyces growth. Greater exchange of specimens (histology slides) among pathologists along the East Coast would also help to resolve any differences in interpretation and in determining the biogeography of the pathogens.
3. Research on molecular methods to detect and quantify Pfiesteria and their

toxins—promising in their early phases—should be accelerated. Molecular and immunological methods to detect and quantify toxins are particularly needed to determine whether there is an ongoing human health risk. It should also be noted that steps should be taken to increase the availability of the toxins for biochemical and biological research.

4. Further biochemical and physiological characterization of the compounds emitted by fish that trigger metamorphosis to toxin-producing stages of Pfiesteria would help us understand the conditions under which toxic outbreaks occur and to determine whether pre-existing lesions increase the likelihood of such outbreaks.
5. The effects of both Pfiesteria toxins and ulcerative mycosis on fish populations should be better assessed. This could be accomplished through research on: the ability of fish to detect toxins; the relative susceptibility of menhaden and other fish species to toxins and fungal infections; specific causes of mass mortality; and the sublethal effects on immunology, health, and populations.

Glossary

Edema. A local or generalized condition of swelling due to fluid accumulation in the tissues. May be caused by fluid imbalance, salt retention, inflammation or chemical substances.
Lesion. Any alteration of a cell, tissue or organ system, microscopic or otherwise, that deviates from normal.

Granuloma. A nodular lesion comprised largely of monocytel macrophages that may or may not be visible to the naked eye. In context, it is a chronic cellular inflammatory response by a vertebrate host to foreign agents that are difficult to kill and degrade, such as certain infectious agents (i.e. bacteria, fungi, parasites).

Hypha (ae). A filamentous, branching, walled, tubular cell filled with protoplasm. A mass of hyphae constitute the thallus (primary body) of a fungus, that is also known as a mycelium.

Ulcer. An open sore or lesion of the skin or mucus membrane, often penetrating through the underlying basement membrane of the affected epithelial cell layers. May be caused by physical trauma, parasites, infectious, toxic or caustic agents, or host response to an inciting agent.

Ulcerative mycosis. An ulcerative disease of fish characterized by deep, penetrating ulcers with mycotic elements and a granulomatous response within the lesions.

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